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PATENT
Attorney Docket No. 016976-000810US

TOWNSEND and TOWNSEND and CREW LLP

Patricia Andrews

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

CHANG et al.

Application No.: 10/766,993

Filed: January 28, 2004

For: SURFACE EXPRESSION OF
BIOLOGICALLY ACTIVE PROTEINS
IN BACTERIA

Customer No.: 20350

Confirmation No. 5009

Examiner: Anoop Kumar Singh

Technology Center/Art Unit: 1632

DECLARATION OF QIANG XU, PH.D.
UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Qiang Xu, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. in the field of Plant Physiology from Kansas State University in 1991. I am currently a Director, Research at Osel, Inc., the assignee of the present patent application. I have been in this position since 2004.

3. I am a named inventor of the present patent application as well as of US Patent Application No. 10/383,834 ("the '834 application"), now US Patent No. 7,179,458. The claims of the present patent application are directed to an *Lactobacillus jensenii* bacterium comprising an expression cassette for expression of a biologically active protein, wherein the protein is linked to a heterologous carboxyl terminal cell wall targeting region as recited in the claims. I understand that the Examiner has rejected the claims as allegedly anticipated by Tagliabue *et al.* (WO 96/11277). A declaration very similar to this one was filed for the '834 application.

4. To my knowledge, as of the priority date of the '834 application (March 8, 2002), no one had reported actual transformation of *L. jensenii*. Indeed, as discussed in detail in the amendment dated August 22, 2005 for the '834 application, there were several reports in the scientific literature that other *Lactobacillus* species could not be successfully transformed. Therefore, one of ordinary skill in the art would not have assumed that any particular protocol was effective to transform *L. jensenii*. Instead, it is my opinion that it was unpredictable as of the priority date of the '834 application what protocol, if any, would be effective to transform *L. jensenii*. As discussed below, it took considerable effort by the inventors of the '834 application to determine conditions that were effective in generating transformed *L. jensenii*.

5. The inventors of the '834 application made initial attempts to transform *L. jensenii* using several published transformation protocols that had been used successfully for other *Lactobacillus* species. Protocols tested included those described in Bringel *et al.*, *Plasmid* 22:193-202 (1989) and Wei *et al.*, *J. Microbiol. Methods* 21:97-109 (1995). In our experiments, neither of these protocols resulted in successful transformation of *L. jensenii*, further demonstrating that it was not a simple or predictable matter to transform *L. jensenii*.

6. An additional electroporation protocol was identified in Luchansky *et al.*, *J. Dairy Sci.* 74:3293-3302 (1991). Luchansky *et al.* teaches transformation of *L. acidophilus* using a specific electroporation protocol involving plasmid ligation mixtures. See, Luchansky *et al.*, page 3296, paragraph spanning left and right columns. It should be noted at this point that to the extent Tagliabue *et al.* described *any* transformation procedure, it involved transformation with ligation mixtures. See, Tagliabue *et al.*, page 11, WO 96/11277). No transformation experiment we have performed using plasmid ligation mixtures or using standard methods (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY) as described in Tagliabue *et al.* have ever resulted in successful transformation of *L. jensenii*. Thus, following the exact protocol and type of DNA (plasmid ligation mixtures) described in Luchansky *et al.* does not result in effective transformation of *L. jensenii*.

7. To achieve transformation of *L. jensenii*, we used intact purified plasmids instead of the ligation mixtures as described in Luchansky *et al.* In addition, instead of using 0.4 cm interelectrode gap cuvettes, per the Luchansky *et al.* protocol, we chose 0.2 cm cuvettes to transform *L. jensenii*. Transformation efficiency of *L. jensenii* was affected by interelectrode gap. *L. jensenii* was cultured to reach 0.7 at OD600 in MRS broth. Cells were washed in sterile distilled H₂O and resuspended. Two hundred microliters of competent cells (about 7×10^8 CFU) were electroporated in 952 mM sucrose and 3.5 mM MgCl₂ with 1 μ g intact purified plasmid DNA, 2.5 kV, and 200 ohms. After electroporation, bacteria were plated on the erythromycin-containing MRS plates for 24 hours. Then, the erythromycin resistant colonies were counted. As shown in Table I below, this cuvette modification resulted in approximately an eight-fold increase in erythromycin resistant colonies compared to the cuvette size used by Luchansky *et al.*

Table I

Interelectrode cuvette (cm)	gap	Time constant	Number of colonies
0.4		4.7	100

0.2	3.76	790
0.1	5.22	0

9. In view of the forgoing, it is clear that merely following protocols described in the prior art for transforming other *Lactobacillus* species was not effective for transforming *L. jensenii*. It is my scientific opinion that transformation of *L. jensenii* was both unpredictable and difficult prior to the significant experimentation we carried out to generate the data included in the '834 application. Therefore, I do not believe it was obvious for one of ordinary skill in the art how to transform *L. jensenii* as of the priority date of the '834 application.

Date: 6/3/08By: Qiang Xu
Qiang Xu, Ph.D.